

any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ TCID₅₀ per dose.

[39 FR 44720, Dec. 27, 1974, as amended at 40 FR 20067, May 8, 1975; 40 FR 23989, June 4, 1975; 40 FR 41089, Sept. 5, 1975; 43 FR 49529, Oct. 24, 1978; 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.311 Bovine Virus Diarrhea Vaccine.

Bovine Virus Diarrhea Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300.

(b) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five bovine virus diarrhea susceptible calves shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individuals serum samples tested. The calves shall be considered susceptible to bovine virus diarrhea virus infection if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test with less than 500 TCID₅₀ of bovine virus diarrhea virus.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 calves to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five calves held as uninjected controls. To confirm the dosage calculations,

five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) At least once during a period 14 to 28 days post-vaccination, individual serum samples shall be collected for virus-neutralization tests from each of the vaccinates. The test virus shall be less than 500 TCID₅₀ of bovine virus diarrhea virus. The white cell count for all vaccinates and controls shall be established at least 3 days just before challenge. Results shall be used in making a determination as prescribed in paragraph (c)(5) of this section.

(4) The vaccinates and the controls shall each be challenged with virulent bovine virus diarrhea virus and observed for 14 consecutive days. The white cell count shall be determined daily on each animal from the second through the eighth day post-challenge. If leukopenia does not develop in at least four of the five controls as compared with the vaccinates, the test shall be considered inconclusive and may be repeated.

(5) If less than 19 of the post-injection serum samples, tested as prescribed in paragraph (c)(3) of this section, show neutralization in all tubes of the 1:8 dilution; or if more than one of the vaccinates exhibits respiratory or other clinical signs of bovine virus diarrhea post-challenge; or both, the Master Seed Virus is unsatisfactory.

(6) A sequential test procedure may be used in lieu of the 20 calf requirement. A beta value of .05 and a tolerance level of .78 shall be required.

(7) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided*, That five of five vaccinates and at least four of the five controls shall meet the criteria prescribed in paragraphs (c)(4) and (c)(5) of this section.

(8) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) Test requirements for release: Each serial and subserial shall meet the applicable general requirements

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prescribed in § 113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested except as prescribed in paragraph (d)(1) of this section. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Purity test.* The test for *Brucella* contamination prescribed in § 113.32 shall be conducted on each batch of primary cells intended for production use.

(2) *Safety test.* The mouse safety test prescribed in § 113.33(a) and the calf safety test prescribed in § 113.41 shall be conducted.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer per dose sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ TCID₅₀ per dose.

[39 FR 44721, Dec. 27, 1974, as amended at 40 FR 20067, May 8, 1975; 40 FR 41089, Sept. 5, 1975; 43 FR 49529, Oct. 24, 1978; 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.312 Rabies Vaccine, Live Virus.

Rabies Vaccine shall be prepared from virus-bearing cell cultures or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300.

(1) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(2) Each lot of Master Seed Virus propagated in tissues or cells of avian

origin shall be tested for pathogens by procedures prescribed in § 113.37.

(3) Each lot of Master Seed Virus propagated in primary cell cultures of mouse or hamster origin or brain tissues of mouse origin shall be tested for lymphocytic choriomeningitis (LCM) virus by the procedure prescribed in § 113.42. If LCM virus is detected, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be studied in each species of carnivore or domesticated wild animal for which the vaccine is specifically recommended to attempt to determine the fate of the vaccine virus. Results shall be considered in evaluating safety of vaccine virus.

(i) Obtain at least 10 unvaccinated animals, negative at 1:2 final serum dilution, of each species in which tests will be conducted. Divide each species into two groups of five animals.

(ii) For each species of animal, inject one group of five animals intramuscularly. Infiltrate a major nerve and the surrounding tissue in each of the five animals in the other group. Use 1.0 ml of high titer virus for each method of administration.

(iii) Observe all animals for signs of rabies until scheduled time to sacrifice. If animals show definite symptoms, sacrifice and check regional lymph nodes, brain, salivary glands, and kidney for rabies virus by injection of suckling mice (not more than 7 days of age). Tissues may be held frozen at -70°C . until suckling mice are available. Inject each mouse in one litter intracerebrally with 0.02 ml of a ground tissue suspension from each organ. Observe mice each day for 21 days. If any mice die, determine if the deaths were due to rabies virus in the brain by a fluorescent antibody test.

(iv) Sacrifice animals that do not show signs of rabies according to the following schedule and check regional lymph nodes, brain, salivary glands, and kidney in suckling mice.

| Route of injection | Days after injection | Number of animals |
|-----------------------|----------------------|-------------------|
| Intramuscularly | 15, 20, 25, 30, 35 | 1 each day. |
| Intraneurally | 3, 6, 9, 15, 30 | 1 each day. |

(5) Each lot of Master Seed Virus shall be tested for safety in at least 10 unvaccinated serologically negative